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CYCLIC VOLTAMMETRY ELECTRODEPOSITION AND MODIFICATION OF SILVER NANOPARTICLES-REDUCED GRAPHENE OXIDE ELECTRODE FOR IMMUNOSENSING

ASILAH JAMIL

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Master of Science

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July 2015

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A silver nanoparticles (AgNPs) and reduced graphene oxide (rGO) nanocomposite electrodeposited on an indium tin oxide (ITO) glass slide using cyclic voltammetry (CV) technique is reported. The modified ITO was used as a platform for the development of a new electrochemical hydrogen peroxide (H_2O_2) biosensor, in which horseradish peroxidase (HRP) tagged antibody acted as a recognition element.

The AgNPs-rGO nanocomposite was synthesized via CV electrodeposition technique in a three-electrode electrochemical cell. Silver-ammonia solution [$Ag(NH_3)_2OH$] was used as a precursor of silver and was prepared by adding ammonia to a silver nitrate ($AgNO_3$) solution until complete absence of precipitate was achieved. The [$Ag(NH_{3)2}OH$] solution was mixed with GO and CV was performed to allow electrodeposition to take place. By applying a negative potential, the GO nanosheets with the absorbed $[Ag(NH_3)_2]^+$ ions were electrodeposited on ITO, simultaneously reducing GO to rGO nanosheets and $[Ag(NH_3)_2]^+$ ions to AgNPs, forming a brown and uniform AgNPs-rGO nanocomposite thin film.

CV and chronoamperometry (CA) techniques were employed in the determination of electrode responses and applicability. The AgNPS-rGO/ITO modified electrode outperformed the bare electrode remarkably, in which the surface area calculated was 0.36 cm^2 compared to bare ITO, which was 0.27 cm^2. The electrochemical conductivity were enhanced significantly by 40-fold, resulting in a notable amplified electrical signal for the detection of H_2O_2. The limit of detection was calculated as 120 µM at the signal-to-noise ratio of 3 with a linear range from 25 µM to 500 µM (R^2 = 0.9944) through CV, while using CA, this modified electrode exhibited a wider linear range from 25 µM to 1355 µM (R^2 = 0.9992) with the detection limit estimated as 10 µM at the signal-to-noise ratio of 3.

Meanwhile, the immunoassay was prepared by immobilizing carcinoembryonic antigen (CEA) between the primary antibody and detection antibody, which was HRP-labelled tagged antibody. The sandwich-type immunoassay represented the sandwich enzyme-linked immunosorbent assay (ELISA) method that is commonly used as a diagnostic tool for cancer detection. The amperometric response of the biosensor was based on the electrocatalytic reduction of H_2O_2 by HRP due its ability to catalyze H_2O_2 reduction process at a lower potential via direct electron transfer. Thus, it was used to enhance response signal and boost analytical sensitivity of the immunoassay.

The CV analysis using the sandwich-type immunoassay configuration resulted in a linear range of 25 µM - 500 µM for the detection of H_2O_2, with a detection limit of 214 µM. Meanwhile, CA offered a wider linear range of 25 µM - 1450 µM, with a detection limit 5.3 µM. Therefore, it had been proven that the current-time response provided a more sensitive measurement towards the detection of H_2O_2.

The resulting biosensor also exhibited excellent stability with a relative standard deviation of 5.0% (n = 3) and remarkable reproducibility of only a 10% decrease in the peak current upon observation after 10 days. The biosensor was also highly selective towards H_2O_2 when compared against various interferences.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGENAPAN VOLTAMMETRI BERKITAR DAN PENGUBAHSUAIAN ELEKTROD NANOPARTIKEL PERAK-GRAFIN UNTUK SENSOR IMUNO

Oleh

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Nanopartikel perak (AgNPs) dan grafin (rGO) yang dienapkan secara voltammetri berkitar (CV) di atas kepingan kaca indium timah oksida (ITO) telah dilaporkan ITO yang telah diubah suai digunakan sebagai platfom untuk mengesan hidrogen peroksida (H_2O_2) di mana antibodi dilabelkan bersama *horseradish peroxidase* (HRP) yang bertindak sebagai elemen pengesan.

Nanokomposit AgNPs-rGO telah disediakan melalui pengenapan secara CV di dalam sistem tiga elektrod. Larutan perak-ammonia $[Ag(NH_3)_2OH]$ digunakan sebagai bahan pemula untuk perak dan disediakan dengan menambah ammonia kepada larutan nitrat perak ($AgNO_3$) sehingga endapan hilang sepenuhnya. Larutan $[Ag(NH_3)_2OH]$ dicampurkan dengan GO dan CV telah dijalankan untuk membenarkan proses pengenapan berlaku. Dengan mengenakan potensi negatif, nanohelaian GO bersama dengan ion-ion $[Ag(NH_3)_2]^+$ yang terserap telah dienapkan ke atas ITO, secara serentak mengurangkan GO kepada nanohelaian rGO dan ion $[Ag(NH_3)_2]^+$ kepada AgNPs, membentuk filem nipis nanokomposit AgNPs-rGO yang berwarna keperangan dan seragam.

CV dan kronoamperometri (CA) telah digunakan untuk mengenal pasti respons dan kebolehgunaan elektrod. Elektrod AgNPS-rGO/ITO yang telah diubah suai mengatasi elektrod tak salut secara luar biasa, dimana luas permukaan yang diperoleh ialah 0.36 cm^2 berbanding ITO tak salut, iaitu 0.27 cm^2. Kealiran elektrokimia meningkat secara mendadak iatu sebanyak 40 kali ganda, menunjukkan kemajuan peningkatan isyarat arus untuk mengesan H_2O_2. Had pengesanan dikira sebagai 120 µM pada 3 isyarat kepada nisbah bunyi dengan julat linear bermula dari 25 µM sehingga 500 µM (R^2 = 0.9944) melalui CV, sementara menggunakan CA, elektrod yang telah diubahsuai

memperlihat julat linear yang lebih luas bermula dari 25 µM kepada 1355 µM (R^2 = 0.9992). Had pengesanan dianggarkan 10 µM pada 3 isyarat kepada nisbah bunyi.

Sementara itu, assai imuno disediakan dengan memegunkan carcinoembryonic antigen (CEA) diantara antibodi utama dengan antibodi pengesan, iaitu antibodi dilabelkan bersama HRP. Assai imuno jenis lapisan mewakili teknik lapisan *enzyme-linked immunosorbent assay* (ELISA) yang biasanya digunakan sebagai alat diagnosis untuk mengesan kanser. Respon amperometri biosensor adalah berdasarkan pengurangan electrokatalistis H_2O_2 oleh HRP disebabkan oleh kebolehannya untuk memangkin proses pengurangan H_2O_2 pada potensi yang rendah melalui pemindahan terus elektron. Oleh itu, HRP digunakan untuk meningkatkan isyarat respon dan melonjakkan kepekaan analisis assai imuno.

Analisis CV menggunakan konfigurasi assai imuno jenis lapisan memberi julat lurus 25 µM - 500 µM untuk pengesanan H_2O_2, dengan had pengesanan 214 µM. Sementara itu, CA menawarkan julat lurus yang lebih luas iatu 25 µM - 1450 µM, dengan had pengesanan 5.3 µM. Oleh itu, dapat dibuktikan bahawa respons arus-masa menyediakan ukuran yang lebih peka terhadap pengesanan H_2O_2.

Biosensor yang dihasilkan mempamerkan stabiliti yang cemerlang dimana sisihan piawai relatif ialah 5.0% (n = 3) dan kebolehulangan yang baik iaitu hanya 10% penurunan puncak arus selepas 10 hari pemerhatian. Biosensor ini juga sangat selektif terhadap H_2O_2 jika dibandingkan dengan pelbagai interferens.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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A	Surface area of the electrode
AgNPs	Silver nanoparticles
APTES	3-aminopropyltriethoxysilane
AuNPs	Au nanoparticles
BSA	Bovine serum albumin
C	Concentration of $K_3[Fe(CN)_6]$
CA	Chronoammperometry
CC	Catechol
CE	Counter electrode
CEA	Carcinoembryonic antigen
CDP	Cyclodextrin
CILE	Carbon ionic liquid electrode
CNTs	Carbon nanotubes
CPE	Carbon paste electrode
CV	Cyclic voltammetry
D	Diffusion coefficient
DPV	Differential pulse voltammetry
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
EDS	Energy dispersive X-ray spectroscopy
EIS	Electrochemical impedance spectroscopy
ELISA	Enzyme-linked immunosorbent assay
EY	Eosin Y
FESEM	Field emission scanning electron microscopy

GCE	Glassy carbon electrode
GO	Graphene oxide
GOx	Glucose oxidase
GS	Graphene sheets
HEH	Hantzsch 1,4-dihydropyridine
HQ	Hydroquinone
HRP	Horseradish peroxidase
HSA	Human serum albumin
I_p	Anodic peak current
ITO	Indium tin oxide
LOC	Lab-on-chip
MIT	Microwaves irradiation techniques
MWCNT	Multi-walled carbon nanotube
N	Number of electron transfers
NAD(P)H	Reduced nicotamine adenine dinucleotide
NHS	N-hydroxysulfosuiccinimide sodium salt
p-ATP	*p*-aminothiophenol
PBS	Phosphate buffer solution
PDA	Polydopamine
PDDA	Poly(diallyldimethylammonium chloride)
PDNPs	Palladium nanoparticles
P-L-His	Poly (L-histidine)
PTH	Poly-thionine
PTHNWs	Poly-thionine nanowires
PtNPs	Platinum nanoparticles

QCM	Quartz microbalance
RE	Reference electrode
rGO	Reduced graphene oxide
SCCA	Squamous cell carcinoma antigen
SERS	Surface enhanced Raman scattering
SPCE	Screen-printed carbon electrode
SPR	Surface-plasmon resonance
TEM	Transmission electron microscopy
TEPA	Tetraethylene pentamine
WE	Working electrode
XRD	X-ray diffraction
υ	Scan rate
$\upsilon^{1/2}$	Square root of the scan rate

CHAPTER 1

INTROUCTION

1.1 Background of Study

Various immunoassay techniques have been developed for the detection of biomolecules like enzyme-linked immunoassay (ELISA), fluoroimmunoassay, radioimmunoassay, electrochemistry, fluorescence, chemiluminescence, surface-plasmon resonance (SPR) and quartz microbalance (QCM) (Li *et al.*, 2011; Su *et al.*, 2011). Earlier, radioimmunoassay was the most frequently used method for biomolecule detection. Unfortunately, radioactive labels are harmful to the operators. Thus, to overcome the harmfulness of radioactive immunoassay, test kits of ELISA and chemiluminescence immunoassay are commonly used in clinical diagnoses (Liu *et al.*, 1985; Mathieu *et al.*, 1989). Although these methods provide more sensitivity and lower detection limit, they are usually sample and time consuming.

To overcome those drawbacks and simultaneously fulfil the demand of miniaturization technologies, the electrochemical immunosensing has become the main analytical technique owing to its sensitivity, simple instrumentation, hassle-free pre-treatment procedures, fast analytical time and precise measurement (Chen *et al.*, 2008; Centi *et al.*, 2009), which makes it specifically suitable for the analysis of a small amount of sample (Gao *et al.*, 2006).An electrochemical biosensor can be constructed by using biological layers and transducer system. Various biological layers such as aptamer (Zuo *et al.*, 2007; Chen *et al.*, 2008; Wang *et al.*, 2010), probe DNA (Sun *et al.*, 2012; Guo *et al.*, 2013), enzymes (Lee *et al.*, 2004; Chen *et al.*, 2010), and antibody and cells (Chen *et al.*, 2006; Zhou *et al.*, 2013) had been applied to interact with the substance to be studied while the transducer system works to recognize the electrochemical reaction and convert the biological signal into electrical signal. Antibody based electrochemical immunosensor like sandwich type immunoassay has shown a great potential in bioanalysis where the detection of biomolecules is through the interaction between an antibody and an antigen (Liu *et al.*, 2013). In sandwich type immunoassay, the secondary antibody molecules are usually tagged with signal tags like enzyme, as a transducer system.

Therefore, the immobilization of antibody is a key step to fabricate immunosensor because antibody, acting as the recognition elements, serves the sites of antibody-antigen reaction. The antigen binding capacity would largely increase with a well-defined oriented antibody surface and performance of the detection system would be enhanced. So the choice of materials in the fabrication of electrochemical immunosensor play an important point to ensure that the antibody could be well-oriented on the surface of electrode and interact well with the antigen (Zhou *et al.*, 2014). This will also contribute to implement a stable and sensitive immunosensor.

Presently, carbon-based material like graphene, has attracted a great attention in research fields. Graphene is a well-known nanomaterial due to its large accessible

surface area, high electrical conductivity and large capacity for immobilizing enzymes in the development of high performance electrochemical immunosensor (Choi *et al.,* 2010). In contrast with the carbon nanotubes (CNTs), graphene possesses likely advantages of low cost, high surface area, excellent conductivity, simple processing and safety (Li *et al.,* 2012). Therefore, diverse strategies have been applied for immobilizing enzymes on graphene and its derivative (Wang *et al.,* 2012). Among the derivatives, reduced graphene oxide (rGO) is a better choice for immunosensing because its electrical conductivity is around 8 times of magnitude larger than that of GO (Zhou *et al.,* 2009). Furthermore, the potent deoxygenating process would benefit the subsequent modification and immobilization of biomolecules. Chemical reduction of GO is a favourable and realistic method to synthesize rGO. Unfortunately, this approach often involves the use of reducing agents which may result in impurity contamination. However, it has been reported that GO can be electrochemically reduced to rGO without involving reducing chemicals, leading to a promising green approach for rGO synthesis (Liu *et al.,* 2014). The electrochemically obtained rGO possesses much better achievement for electrochemical applications than the chemically reduced counterpart (Shao *et al.,* 2010). rGO as an electrode material can be functionalized to promote electron transfer between the electroactive species and the electrode to afford a novel method for fabricating immunosensor (Yu *et al.,* 2012)

Nanocomposite materials consist of rGO and metal nanoparticles have received much interests among researchers becaused they not only enhance the properties of rGO but also contribute to developing synergistic composite materials that are able to yield high conductivity and excellent electrochemical performance (Huang *et al.,* 2011). Inexpensive silver nanoparticles (AgNPs), which hold high catalytic activity (Rashid and Mandal 2007), notable optical properties and strong surface enhance Raman effect (Nie and Emory 1997), have received great attention in metal based nanomaterials for the application of ultrasensitive chemicals and biological molecules detection (Abdulrahman 2012) as compared to other metals. Decorating silver nanoparticles on rGO can be accomplished by various methods, however, electrochemical method is a favourite choice owing to a single-step strategy, cost effectiveness and eco-friendliness as it does not involve any reducing agent (Moradi Golsheikh *et al.,* 2013). By the same token, as a consequence of large surface area and high surface energy offered by the nanocomposite, antibody antigen effectively absorbs on the nanocomposite, increasing both enzyme stability and immobilization capability (Liu *et al.,* 2011)Presently, various types of electrodes had been used for electrochemical detection of biomolecules. Glassy carbon electrode (GCE), gold electrode, platinum electrode and graphite electrode are among the electrodes that had been modified to fulfil the demand for electrochemical sensing (Ruzgas *et al.,* 1995; Shen and Liu 2007; Goyal *et al.,* 2008; Lin *et al.,* 2012; Yu *et al.,* 2012). However, the use of indium tin oxide (ITO) is emphasized as it is one of the important materials in the fabrication of the electrodes for electrochemical sensing. Its advantages like wide potential window, high optical transparency and low capacitive current make it highly favoured in microfluidic (Kadri *et al.,* 2008). Microfluidic is a field that is now routinely used in several commercial applications like lab-on-a-chip (LOC) systems that allow precise manipulation of fluids that is usually small volumes in order of micro- and pico-litres. As it also offers the miniaturization of systems, it is becoming increasingly attractive in chemistry and biochemistry as it is normally employed in these laboratories (Chrimes *et al.,* 2013). In addition, the ease of micropatterning of ITO, which is practical in the fabrication of

microelectrodes for handling small amount of samples, fulfils the properties for microfluidic system (Kadri *et al.*, 2008).

1.2 Problem Statement

Carbon nanotubes (CNTs) have been the most extensively used filler for the hybridization for the purpose of enhancement due to their unique structural, mechanical and electronic properties. However, their fundamental issues like poor dispersion and lack of interfacial bonding limit the real applications of CNTs. The introduction of graphene is reported to present more advanced properties and is likely to display fewer of the weakness that plagued CNTs. Its two dimensional single atomic planar sheet of sp^2 bonded carbon offers a unique two-dimensional environment for electron transport and swift heterogeneous electron transfer at their edges. In addition, the easy dispersion of graphene in a variety of solvents after its conversion to GO, helps in improving interfacial interactions with the other matrix.

Noble metal nanoparticles like Au, Pt and Pd are of great interest because of their extraordinary properties and have been widely used in various applications. However, the fundamental issues of these metals like high cost and difficultly in handling limit their novel applications. Presently, inexpensive AgNPs, which hold high catalytic activity, notable optical properties and strong surface enhanced Raman effect, have received great attention in metal-based nanomaterials for the detection of ultrasensitive chemicals and biological molecules as compared to other metals.

Most of the chemically synthesized graphene materials contain principle element oxygen at its edge or surface, which greatly influences the electrochemical performance of graphene in terms of the heterogeneous electron transfer rate and they are called reduced graphene oxide (rGO). Presently, the rGO has been prepared by various techniques. However, those approaches often employ explosive and poisonous reducing agents and also time consuming. Thus, the electrochemical reduction of GO is a better choice since it offers only a single step and is free from reducing agent, making it safe to the environment.

The determination of H_2O_2 is greatly important as it has been involved in many chemical, biological, pharmaceutical, clinical, environmental and food processes. Numerous approaches have been employed to detect H_2O_2. However, those reported methods such as titrimetry, fluorimetry, spectrophotometry, and chemiluminescence are non-preferable due to their high cost and time consuming properties. The electrochemical method based on enzyme-based biosensor is an alternative as it offers low cost, high sensitivity, portability, short analytical time measurement of H_2O_2.

1.3 Objectives of the study

1. To prepare silver nanoparticles-decorated reduced graphene oxide via cyclic voltammetric electrodeposition method.

2. To assess the biocompatibility between the nanocomposite and immobilized biomolecules.

3. To evaluate the electrocatalytic reduction of H_2O_2 using HRP-tagged antibody for immunoassay with improved linear range and sensitivity.

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